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Protective Effect of Royal Jelly on Some Hematologic Parameters Against Sodium Arsenite Toxicity: A Study in Rats

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Keywords
Royal jelly, sodium arsenite, hematological parameters, heavy metal toxicity

Abstract: This study examined the effect of royal jelly on some hematological values against sodium arsenite ($NaAsO_2$)-induced toxicity in rats. Royal jelly is a known functional bee product; therefore, its potential to protect against these effects is of great importance. In this study, the effects of royal jelly on hematologic changes were investigated. In the study, 35 healthy Wistar albino male rats weighing 250–300 g and 12–13 weeks old were used. Rats were administered daily by gavage for 14 days. WBC, LYM, MID, GRA, RBC, HGB, MCHC, MCH, MCV, HCT, PLT, MPV, and PDW values were measured in blood samples. When the measured values were analyzed, the MID, RBC, MCHC, MCH, MCV, and PDV values did not show statistical significance in all experimental groups (P>0.005). However, significant decreases in %LYM, %MID, HCT, and PLT values and increases in WBC, GRA, and %GRA counts were observed in all groups ($P < 0.005$). This review shows that royal jelly has a potential effect on the hematological and immune systems against toxicity caused by harmful heavy metals such as arsenic. In particular, further research is required to identify the most effective dosage of royal jelly. The results highlight the protective potential of royal jelly against toxicity, which requires further investigation.

Arı Sütünün Sodyum Arsenit Toksisitesine Karşı Bazı Hematolojik Parametreler Üzerindeki Koruyucu Etkisi: Ratlarda Bir İnceleme

Anahtar Kelimeler
Arı sütü, sodyum arsenit, hematolojik parametreler, ağır metal toksititesi

Öz: Bu çalışma, ratlarda sodyum arsenit ($NaAsO_2$) kaynaklı toksisiteye karşı arı sütünün bazı hematolojik değerlere olan etkisini incelemektedir. Arı sütü, bilinen fonksiyonel bir arı ürünüdür ve bu nedenle, bu etkilere karşı koruma sağlama potansiyeli büyük önem taşımaktadır. Çalışmada, hematolojik değişiklikler üzerinden arı sütünün etkileri araştırılmaktadır. Çalışmada, 250-300 gram ağırlığındaki 12-13 haftalık 35 sağlıklı Wistar albino erkek sıçan kullanıldı. Sıçanlara 14 gün boyunca gavaj yolu ile günlük olarak uygulama yapıldı. Kan örneklerinde WBC, LYM, MID, GRA, RBC, HGB, MCHC, MCH, MCV, HCT, PLT, MPV ve PDW değerleri ölçüldü. Ölçülen değerler incelendiğinde, MID, RBC, MCHC, MCH, MCV ve PDV değerleri tüm deney gruplarında istatistiksel olarak anlamlılık göstermedi (P>0.005). Ancak, %LYM, %MID, HCT ve PLT değerlerinde tüm gruplarda önemli düşüşler, WBC, GRA ve % GRA sayılarında ise artışlar gözlendi ($P < 0.005$). Bu inceleme, arı sütünün arsenik gibi zararlı ağır metallerin neden olduğu toksisiteye karşı hemotolojik sistem ve bağışıklık sistemi üzerinde potansiyel bir etkisi olduğunu göstermektedir. Özellikle, arı sütünün en etkili dozajının belirlenmesi için daha fazla araştırmaya ihtiyaç vardır. Sonuçlar, arı sütünün toksisiteye karşı koruyucu potansiyelini vurgulamakta olup, bu potansiyelin daha fazla araştırılması gerekmektedir.

1. INTRODUCTION

Royal jelly is an extremely valuable bee product that provides numerous benefits in terms of nutrition and bee diversity. Interestingly, although all bees have the same genetic makeup, queen bees, and worker bees differ significantly in their physiology and morphology - this difference can be attributed to the exclusive consumption of royal jelly by queen bees [1]. Royal jelly is known as a nutrient considered extremely valuable for health and is used as a cosmetic and dietary supplement, with the belief that its effects on bees will have similar effects on humans. Royal jelly is notable for its unique biochemical composition, a special secretion produced by bees of the Apis mellifera species. Its proteins, lipids, vitamins, minerals, and bioactive peptides support the potential health benefits of these components on the body. The health benefits of royal jelly have been studied in various aspects such as immune system modulation, antioxidant effects, maintenance of skin health, and alleviation of aging processes [2]. The pharmacological properties of royal jelly have been characterized by a range of biological activities observed in experimental animals. These activities include growth-promoting, antiseptic, antitumor, antibacterial, antioxidant, immunomodulatory, antihypertensive, and anti-inflammatory activities. In addition, royal jelly is believed to have anti-aging, healing, hypoglycemic (anti-diabetic) and anti-cancer properties. Biologically, the special components in royal jelly form the basis for these various pharmacological effects. For example, proteins may support the mechanisms behind growth-promoting effects, while antioxidants may be effective in reducing cellular damage. The immunomodulatory effects of royal jelly can be explained by exerting a regulatory effect on the immune system [3].

Heavy metals are elements that have a high atomic mass, metallic properties, a density greater than 5 g/cm3, and are chemically stable and widely distributed in nature. Heavy metals are elements that can enter the body through the mouth, respiratory tract, and skin. These metals can have toxic effects even at low concentrations, meaning they can be harmful even in very small amounts. These dangerous properties arise because heavy metals are easily absorbed in the body, but cannot be effectively eliminated from the body. In living organisms, heavy metals can accumulate and concentrate. These metals can cause serious health problems when they exceed a certain threshold dose. These problems can include poisoning, organ damage, and even death [4]. Although industrialization has brought many economic and technological benefits to humanity, it is also an important factor that has led to an increase in environmental problems. This process has led to the emergence of environmental problems such as heavy metal pollution and these problems have reached great dimensions over time. With the increase in industrial activities around the world, the number of metal elements exposed to the external environment has also increased. More than 35 of these metals have been detected in the external environment and 23 of these elements are heavy metals. Heavy metals are elements found in nature and can be released into the environment as a result of human activities such as various industrial processes, mining activities, energy production, and vehicular traffic. Many of these metals can enter the body through the mouth, respiratory tract, and skin and therefore have the potential to harm human health and the environment [5].

Arsenic (As) is one of the twenty most common elements in the earth's crust and is classified as a metal and nonmetal element in terms of its chemical properties. Because of this property, arsenic is also called a metalloid. Arsenic occurs naturally in different parts of the Earth's crust and is present as part of various minerals and ores. Industrial and agricultural practices can lead to the release of arsenic into the environment, which can cause arsenic contamination in water sources and soils. Arsenic can pose a significant hazard to human health. Exposure to high levels of arsenic can cause serious health problems and have carcinogenic effects. It is therefore important to monitor and control arsenic contamination. Arsenic, as an element released into the environment by both natural processes and human activities, is of great importance in the fields of environmental chemistry and health sciences. Its environmental and human health impacts are the focus of scientific research and it is important to learn more about arsenic from an environmental and human health perspective [6].

Hematology is an intriguing field of research that focuses on the cellular components of blood, including erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (thrombocytes), and studies the relationship between the physiology and pathology of these components. By understanding the normal functioning of these cells, hematologists can identify and treat a range of blood-related health conditions. Blood studies provide deep knowledge about the functions and changes of these cellular components, which contributes significantly to our understanding of the functioning of the human body. Hematology sheds light on understanding and treating blood diseases, immune system problems, and other health conditions related to the circulatory system. Blood analysis and the study of cellular components help diagnose diseases early, formulate treatment plans, and monitor patients' health [7].

Hematological parameters such as red blood cell count, white blood cell count, and hemoglobin concentration are widely used as clinical indicators of health and disease. These parameters are a reflection of the biochemical components and cellular structure of the blood and provide important information about the overall health status of the body. Red blood cell count correlates with hemoglobin levels, reflecting the body's capacity to transport oxygen. Hemoglobin is an important molecule that determines the blood's ability to carry oxygen. Therefore, red blood cell count and hemoglobin concentration are critical in diagnosing and monitoring oxygen transport problems such as anemia. White blood cell count reflects the activity of the immune system and is used to monitor various health problems, such as infections and inflammatory conditions. The number and types of white blood cells help determine the type and severity of infections. These hematologic parameters play an important role in establishing medical diagnoses and treatment plans. They are also regularly monitored to monitor patients' health status and assess their response to treatment. Thus, hematologic parameters are an indispensable tool for disease diagnosis and treatment management in the medical field [8]. Leukocytes, also known as white blood cells, are essential in the immune system and are divided into two main categories: granulocytes and agranulocytes. Granulocytes, with small particles, are the first line of defense against diseases, while agranulocytes, monocytes, and lymphocytes are the immune system's clean-up crew and direct long-term immune responses, creating specialized defense mechanisms like antibodies [9]. Leukocytosis is a condition where the number of white blood cells in the body exceeds normal levels, often indicating health issues and being linked to the immune system. It is crucial to identify and treat the cause of leukocytosis to ensure proper health outcomes [10]. White blood cells (WBCs) are vital in our body's defense system, constantly active in the bloodstream. They protect against threats and are activated by pathogens or harmful substances. This self-defense mechanism increases leukocyte numbers, preventing infections and maintaining health. Leukocytosis, a natural defense mechanism, can signal health problems like infections. The WBC value includes neutrophils, eosinophils, lymphocytes, monocytes, atypical leukocytes, or any combination thereof [12]. Lymphocytes, agranulocytes, are white blood cells that play a crucial role in the immune system. They detect and respond to antigens, forming an essential part of the body's defense mechanism against infections. Lymphocytes identify foreign organisms or pathogens, activating other immune system components to protect against infections. Their role is vital in maintaining the health of the immune system and protecting the body from harmful agents [13]. Monocytes, agranulocytes, are white blood cells that play a crucial role in the body's immune system. They detect, engulf, and eliminate foreign substances through phagocytosis, acting as the first line of defense against infectious agents in rats. Monocytes recognize harmful organisms or foreign substances and neutralize them, making them a vital component of the immune response. Their role is vital for the body's health and the effectiveness of the immune system, making their function crucial [14]. Platelets, a small, reactive blood cell, play crucial roles in fibrosis and blood clotting processes. They undergo changes when stimulated by inflammatory cytokines and classical activators, altering cell shape, forming pseudopodia, and releasing cytoplasmic granules. Platelets accumulate rapidly at damaged sites and form the first line of defense, making their multifaceted role critical for maintaining body health [15].

Previous studies on the effect of arsenic on the hematopoietic system indicate that exposure to high doses of arsenic can affect and alter the hematopoietic system and these hematopoietic parameters [16]. In blood samples, blood leukocyte parameters, blood erythrocyte-hemoglobin, hematocrit parameters, platelet-related parameters WBC, LYM, MID, GRA, RBC, HGB, MCHC, MCH, MCV, HCT, PLT, MPV, and PDW values were examined and the potential effect of royal jelly on the hematological system and immune system against the toxicity caused by sodium arsenic was evaluated.

2. MATERIAL AND METHOD

2.1. Animal Selection and Permissions: Thirty-five male Wistar albino rats weighing 250-300 grams and 12-13 weeks old were used for the experiment. Animal experiments were performed at Bingöl University Animal Experiment Center. The permission for the experiment was obtained from Bingöl University Animal Experiments Local Ethics Committee with the decision number 07/01 dated 09.12.2021.

2.2. Animal Care: Rats were kept in a control room with a temperature of 24-25°C and a twelve-hour dark photoperiod (19:00-07:00 dark; 07:00-19:00 light). Animals were given unlimited access to water and standard food. Rats were rested and acclimatized for one week while they were maintained in cages.

2.3. Experimental Groups: Rats were randomly divided into five different groups:

a) Control Group (C): Rats were given 0.5 cc saline by gavage for 14 days.
b) Royal Jelly Group (R): Rats were given 1 g royal jelly at a dose of 200 mg/kg, dissolved in 10 cc distilled water, and given 0.5 cc by gavage for 14 days (Solution was prepared daily).
c) $NaAsO_2$ Group (Arc): Rats were given $NaAsO_2$ at a dose of 10 mg/kg for 14 days, 1.250 g $NaAsO_2$ was dissolved in 250 cc distilled water to form a stock solution and given 0.5 cc by gavage.
d) $NaAsO_2$ + Royal Jelly 100 mg/kg Group (Arc-R-100): Rats were given $NaAsO_2$ at a dose of 10 mg/kg from the stock solution orally for 14 days. Royal jelly was administered 30 minutes before $NaAsO_2$ administration at a dose of 100 mg/kg by dissolving 0.250 g royal jelly in 5 cc distilled water and administering 0.5 cc by gavage (Solution was prepared daily).
e) $NaAsO_2$ + Royal Jelly 200 mg/kg Group (Arc-R-200): Rats were given $NaAsO_2$ at a dose of 10 mg/kg from the stock solution orally for 14 days. Royal jelly was administered 30 minutes before $NaAsO_2$ administration at a dose of 200 mg/kg by dissolving 1 g royal jelly in 10 cc distilled water and administered by 0.5 cc gavage.

2.4. Sample Collection and Determination of Hematologic Parameters

One day after the end of the study period, animals were decapitated under sevoflurane anesthesia, and blood samples were collected in 3 ml EDTA vacuum tubes. Blood samples were analyzed for White Blood Cell Count (WBC), Lymphocyte (LYM), Monocyte (MID), Granulocyte (GRA), Erythrocyte (RBC), Hemoglobin (HGB), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Hematocrit (HCT), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) parameters were analyzed. Blood samples were read on BeneSphera™ Brand Hematology Analyzer. Analyses were performed using standard methods [17].

2.5. Statistical Analysis

Differences between the experimental groups were determined using IBM SPSS Statistical software (Version 27) (USA). Statistical evaluation of the data was performed using One-Way ANOVA and Bonferroni test as post hoc. Values were considered statistically significant at $P < 0.005$.

3. RESULTS

WBC, LYM, MID, GRA, RBC, HGB, MCHC of (C), (R), (Arc), (Arc-R-100), (Arc-R-200) groups, MCH, MCV, HCT, PLT, MPV, PDW values are given in Table 1. Statistical comparison was made between the (C) group, (R) group, (Arc) group, (Arc-R-100) group, and (Arc-R-200) group. Values are the mean values of seven separate observations with a standard deviation. Statistically significant values are marked in the table. In all experimental groups, MID, RBC, MCHC, MCH, MCV, PDV hematologic values were statistically insignificant in all groups.

3.1. When the control group and other groups were compared statistically; the following hematologic values were found significant (P<0,005) (Table 1).

i. Between (C) + (R) group; A statistically significant difference was found in %LYM value. This indicates that royal jelly may affect the percentage of lymphocytes.
ii. Between (C) + (Arc) group; Statistically significant differences were found in WBC, GRA, %LYM, %MID, %GRA, PLT, HCT values. This indicates that arsenic exposure may affect these parameters.
iii. Between (C) + (Arc-R-100) group; Significant differences were found in WBC, GRA, %LYM, %MID, %GRA values. This indicates that there were significant differences in the values of white blood cells (WBC), granulocytes (GRA), the percentage of lymphocytes (%LYM), the percentage of medium-sized cells (%MID), and the percentage of granulocytes (%GRA) between the group treated with 100 mg/kg royal jelly (Arc-R-100) and the control group. These results indicate that royal jelly has a protective effect against arsenic toxicity.
iv. Between (C) + (Arc-R-200) group; Significant differences were found in %LYM, %MID, %GRA, HCT values. This indicates that there were significant differences in the percentage of lymphocytes (%LYM), the percentage of medium-sized cells (%MID), the percentage of granulocytes (%GRA), and hematocrit (HCT) values between the group treated with 200 mg/kg royal jelly (Arc-R-200) and the control group ©. These results may indicate that royal jelly has a protective effect against arsenic toxicity.

3.2. When the royal jelly group was compared with the other groups; the following hematologic values were found significant (P<0,005) (Table 1).

i. Between (R) + (Arc) group; A significant difference was found in the PLT value. This indicates that arsenic exposure may affect the platelet count.
ii. Between (R) + (Arc-R-100) group; Significant differences were found in WBC and LYM values. This indicates that royal jelly may affect these parameters.
iii. Between (R) + (Arc-R-200) group; A significant difference in HGB value was found. This indicates that royal jelly may affect hemoglobin levels.

3.3. When the arsenic group and other groups were compared statistically; the following hematologic values were found significant (P<0,005) (Table 1).

i. Between (Arc) + (Arc-R-100) group, PLT
ii. Between (Arc) + (Arc-R-100) group; PLT, MPV

Significant differences were found in PLT and MPV values. This indicates that royal jelly may modify the effect of arsenic exposure on these parameters.

3.4. When $NaAsO_2$ + royal jelly 100 mg/kg group and $NaAsO_2$ + royal jelly 200 mg/kg group were compared statistically; the following hematologic values were found significant (P<0,005) (Table 1).

Between (Arc-R-100) + (Arc-R-200); the HGB value (P<0,005) was found significant, while other hematological values (P>0,005) were found statistically insignificant. This indicates that different doses of royal jelly may have different effects on hemoglobin levels.

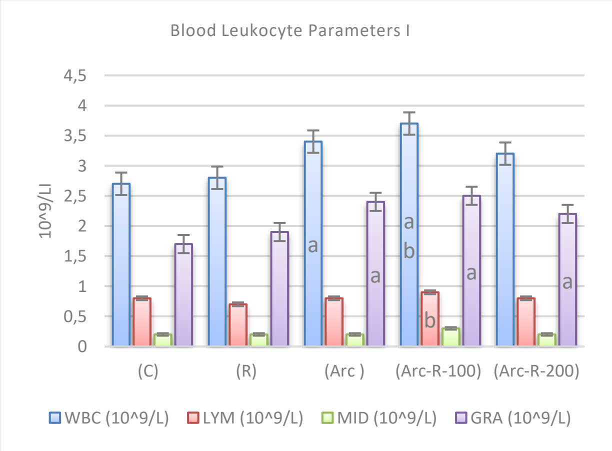

Figure 1. Blood Leukocyte Parameters in Control and Experimental Groups a- The difference compared to the control group is statistically significant (P<0.005) b- The difference according to the royal jelly group is statistically significant (P<0.005)

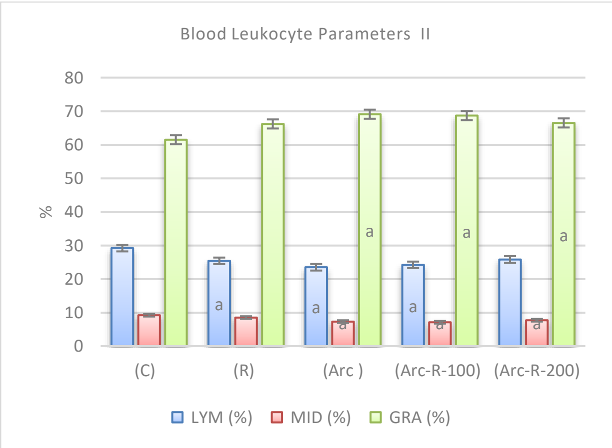

Figure 2. Percent Blood Leukocyte Parameters in Control and Experimental Groups a- The difference compared to the control group is statistically significant (P<0.005)

When the blood leukocyte parameters in the control and experimental groups were examined, the effects of different treatments (Control Group, Royal Jelly Group, $NaAsO_2$ Group, and different concentrations of Royal Jelly & $NaAsO_2$) on blood leukocyte parameters were revealed. Compared to the control group, significant changes were observed in the blood leukocyte parameters of the royal jelly group and $NaAsO_2$ Group. In particular, WBC (10^9/L) values were significantly higher in the Royal Jelly Group compared to the control group (P<0.005). Furthermore, a significant decrease was observed in LYM (%) and GRA (%) values in the $NaAsO_2$ group compared to the control group (P<0.005) (Figure 1-2).

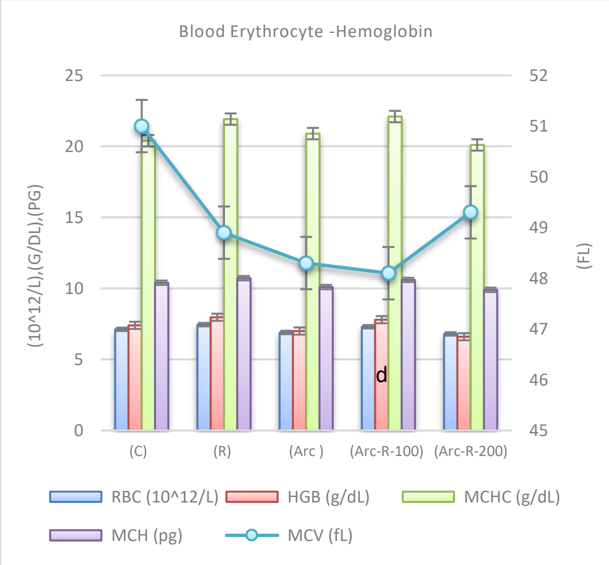

Figure 3. Blood Erythrocyte-Hemolobin Parameters in Control and Experimental Groups d- There is a significant difference between the 100 mg/kg group of royal jelly and the 200 mg/kg group of royal jelly (P<0.005)

When the blood erythrocyte-hemoglobin parameters in the control and experimental groups were examined, the effects of different treatment groups (control, royal jelly, $NaAsO_2$, and royal jelly + $NaAsO_2$ combinations) on RBC, HGB, MCHC, MCH, and MCV values were shown. A significant difference was found between the 100 mg/kg royal jelly group and the 200 mg/kg royal jelly group in the HGB parameter (P<0.005). The findings indicate that royal jelly dosage has a significant effect on blood erythrocyte-hemoglobin parameters. In particular, a significant difference was found between 100 mg/kg and 200 mg/kg royal jelly doses, indicating that royal jelly has a dose-dependent effect on these parameters (Figure 3).

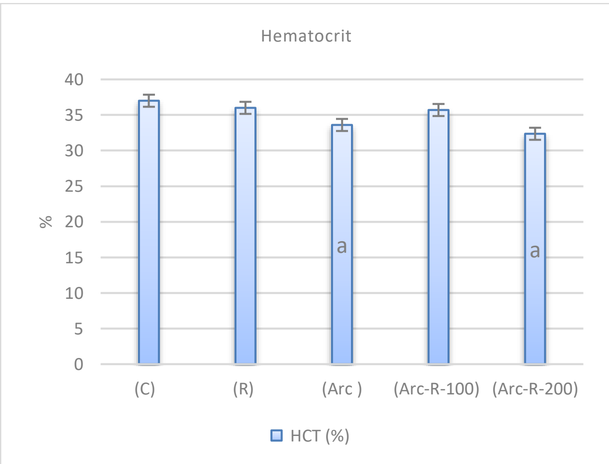

Figure 4. Hematocrit Parameter in Control and Experimental Groups a- The difference compared to the control group is statistically significant (P<0.005)

The control group and Royal Jelly Group had higher hematocrit levels than the $NaAsO_2$ -exposed groups, while the hematocrit levels decreased in the $NaAsO_2$ -exposed groups. However, when royal jelly was given before $NaAsO_2$ exposure, there was a partial improvement in hematocrit levels. This suggests a potential protective effect of royal jelly against $NaAsO_2$ toxicity. These findings are statistically significant (P<0.005)

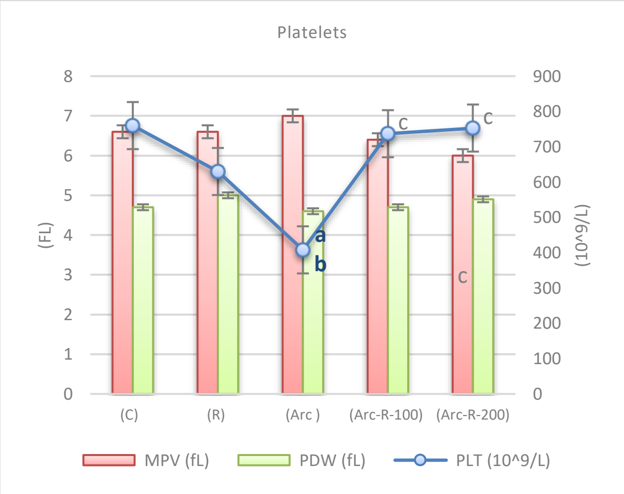

Figure 5. Platelet-Related Parameters in Control and Experimental Groups a- The difference compared to the control group is statistically significant (P<0.005) b- The difference according to the royal jelly group is statistically significant (P<0.005) c- The difference compared to the arsenic group is statistically significant (P<0.005)

There was no statistically significant difference in PDW values between any groups. The decrease in the MPV value indicates a decrease in the size of platelets. The decrease in MPV values compared to the arsenic group may indicate that royal jelly may have a protective effect against arsenic toxicity. Arsenic can impair the formation and function of blood cells. This can lead to changes in the size and number of platelets. However, a reduction in MPV values was observed in the royal jelly group, suggesting that royal jelly may mitigate these negative effects. Compared to the control group and the royal jelly group, a significant decrease in PLT levels was observed in the $NaAsO_2$ group. However, in the royal jelly-treated groups Arc-R-1 and Arc-R-2, PLT levels were higher than in the $NaAsO_2$ group. This suggests that royal jelly has a protective effect against $NaAsO_2$ -induced toxicity (Figure 5).

Table 1. Some Hematological Parameters in Control and Experimental Groups a- The difference compared to the control group is statistically significant (P<0.005) b- The difference according to the royal jelly group is statistically significant (P<0.005) c- The difference compared to the arsenic group is statistically significant (P<0.005) d- There is a significant difference between the 100 mg/kg group of royal jelly and the 200 mg/kg group of royal jelly (P<0.005)

Hemogram	**Control Group +SD**	**Royal Jelly Group +SD**	**$NaASO_2$ Group +SD**	**100 mg/kg Royal Jelly + $NaASO_2$ Group +SD**	**200 mg/kg Royal Jelly+ $NaASO_2$ Group +SD**
WBC (10^9/L)	2,7 ± 0,13	2,8 ± 0,17	3,4 ± 0,43 ^{a}	3,7 ± 0,21 $^{a b}$	3,2 ± 0,36
LYM (10^9/L)	0,8 ± 0,06	0,7 ± 0,00	0,8 ± 0,10	0,9 ± 0,02 ^{b}	0,8 ± 0,13
MID (10^9/L)	0,2 ± 0,05	0,2 ± 0,04	0,2 ± 0,05	0,3 ± 0,06	0,2 ± 0,04
GRA (10^9/L)	1,7 ± 0,10	1,9 ± 0,15	2,4 ± 0,30 ^{a}	2,5 ± 0,55 ^{a}	2,2 ± 0,29
LYM (%)	29,2 ± 1,50	25,4 ± 2,40 ^{a}	23,5 ± 0,68 ^{a}	24,2 ± 1,16 ^{a}	24,6 ± 3,74 ^{a}
MID (%)	9,2 ± 0,60	8,5 ± 1,07	7,3 ± 0,42 ^{a}	7,1 ± 0,74 ^{a}	7,7 ± 0,40 ^{a}
GRA (%)	61,5 ± 2,03	66,2 ± 3,36	69,1 ± 0,79 ^{a}	68,7 ± 1,90 ^{a}	67,6 ± 4,10 ^{a}
RBC (10^12/L)	7,12 ± 0,68	7,45 ± 0,34	6,9 ± 0,35	7,3 ± 0,16	6,8 ± 0,49
HGB (g/dL)	7,4 ± 0,35	7,97 ± 0,68	7,0 ± 0,62	7,8 ± 0,15 ^{d}	6,6 ± 0,64 ^{b}
MCHC (g/dL)	20,4 ± 1,46	21,92 ± 1,65	20,9 ± 1,37	22,1 ± 0,76	20,1 ± 1,12
MCH (pg)	10,4 ± 1,0	10,72 ± 0,76	10,1 ± 0,81	10,6 ± 0,26	9,9 ± 0,80
MCV (fL)	51,0 ± 1,79	48,9 ± 1,75	48,3 ± 1,12	48,1 ± 0,66	49,3 ± 1,34
HCT (%)	37 ± 2,50	36 ± 1,10	33,6 ± 2,18 ^{a}	35,7 ± 0,39	32,36 ± 1,14 ^{a}
PLT (10^9/L)	760 ± 46,57	630 ± 115,51	408 ± 105,26 $^{a b}$	737 ± 47,75 $^{a c}$	753 ± 82,59 ^{c}
MPV (fL)	6,6 ± 0,19	6,6 ± 0,44	7,0 ± 072	6,4 ± 0,08	6 ± 0,28 c
PDW (fL)	4,7 ± 022	5 ± 0,26	4,6 ± 0,92	4,7 ± 0,14	4,9 ± 0,12

4. DISCUSSION

Royal jelly is a natural product produced by the hypopharyngeal and mandibular glands of worker bees and has been widely used in alternative medicine for centuries. Rich in ingredients, royal jelly is abundant in proteins, lipids, amino acids, minerals, and vitamins. It also contains bioactive compounds such as peptides, flavonoids, fatty acids, and phenolic acids [18]. Heavy metal pollution is a serious concern for the environment and human health. However, some supplements may play a potential role in reducing the harmful effects caused by heavy metals in the body. These supplements may help prevent the accumulation of heavy metals in the body or reduce their toxicity [19]. Thanks to its antioxidant properties, royal jelly can reduce cellular oxidative stress, regulate the immune system, suppress inflammation, and reduce the effects of aging. It can also fight germs with antibacterial and disinfectant properties, have anti-cancer potential, lower blood pressure by dilating blood vessels, improve cholesterol levels, protect liver health, and inhibit tumor development. Royal jelly is, therefore, a valuable natural supplement that offers many important health benefits [20]. Arsenic is one of the heavy metals found in nature, and humans can be exposed to it through inhaled air, consumption of food and water, and skin contact. Once in the body, it can affect many organ systems, including the skin, respiratory, cardiovascular, immune, reproductive and urinary systems, digestive, and nervous systems [21]. Hematology and biochemical indicators are reliable parameters for assessing the health status of humans and animals suffering from arsenic poisoning. These indicators are important for monitoring the effects of arsenic exposure and determining the degree of toxicity. Hematological parameters provide information on blood composition and circulatory system health, while biochemical indicators assess body functions and organ systems. These parameters play a critical role in detecting arsenic poisoning and guiding treatment processes [22]. This research was conducted to predict the extent to which the harmful effects caused by arsenic exposure can be reduced by royal jelly. This evaluation included hematological parameters, as these parameters can help determine the degree of harmful effects on the blood components of animals. The monitoring of hematological parameters was used as an important tool to evaluate the protective effect of royal jelly against arsenic and arsenic-induced toxicity and to provide important information in this regard. Some values from studies on sodium arsenite exposure in rats are as follows. Kumari et al. [23] observed a significant decrease in WBC, RBC, Hb, Hct, MCV, and MCH in rats exposed to sodium arsenite. However, MCHC remained the same. Sharma and Rani [24] reported a decrease in RBC, Hb, HCT, and PLT and an increase in WBC values. Ola-Davies et al. [11] found significant decreases in PCV, Hb, and RBC, increases in WBC, platelets, lymphocytes, and eosinophils, and decreases in neutrophils and monocytes. The reasons for the differences in the data obtained from different studies may be various environmental factors such as application method, dose, and application time.

Granulocytes are white blood cells that play an important role in the immune system's response to conditions such as infection, allergies, and asthma. Granulocytes are the most common type of white blood cells and are usually divided into three main categories: neutrophils, eosinophils, and basophils. These cells play important roles in various immune responses [25]. Kumari et al. found that granulocyte values were significantly higher in rats exposed to sodium arsenite compared to the control group. In our study, a significant increase in granulocytes was observed in rats exposed to sodium arsenite compared to the control group ($P<0.005$). This increase was not significant in the royal jelly group ($P>0.005$). The results suggest that royal jelly, instead of lowering granulocyte levels, may stimulate more granulocyte production by making the immune system more active.

White blood cells or leukocytes play a vital role in phagocytosis, immunity, and defense against infection [26]. Increased white blood cell counts are known to be a normal reaction to foreign bodies [27]. Ewere et al. [28] found that WBC values in rats exposed to sodium arsenite increased significantly compared to the control group. In our study, an increase in WBC levels was observed. Especially in Arc and Arc-R-100 groups, this increase in WBC levels was found to be statistically significant. In addition, it was observed that this increase became insignificant compared to the control group when the amount of royal jelly in the Arc-R-100 group was increased to 200 mg/kg in the Arc-R-200 group and the values approached the control values. This shows that the amount of royal jelly can affect WBC levels and can be considered an indicator of recovery. These results indicate that royal jelly has a protective effect against sodium arsenite exposure and has a positive effect on the immune system.

Lymphocytes are an important part of the immune system and initiate immune responses by sensing pathogens entering the body. A low lymphocyte count can cause the body to become vulnerable to germs and diseases. This can reduce the body's ability to maintain an effective defense mechanism against harmful microorganisms. Individuals with a low lymphocyte count may therefore have an increased risk of infection and may need to take extra precautions [29]. Ola-Davies et al. found that LYM values increased in rats exposed to sodium arsenite compared to the control group. Our research findings show a significant increase in LYM levels between the R group and the combination groups Arc-R-100 and Arc-R-200. This suggests that royal jelly may have a positive effect on the immune system by increasing the number of lymphocytes. Furthermore, the lymphocyte percentage values were statistically significantly increased in the arsenic-exposed groups unlike the other groups ($P<0.005$). This supports the hypothesis that royal jelly has the potential to increase the HCT count of other shaped elements in the bloodstream and suggests that royal jelly may have positive effects on the immune system and provide a protective effect against toxicity.

Monocytes play a critical role in the immune system, having the ability to transform into macrophages and dendritic cells when needed to fight against infections. When they move into infected tissues, they help to activate the adaptive immune system by initiating an inflammatory response. The versatility of monocytes is considered a vital component of the immune system's defense against harmful pathogens [9]. Ola-Davies et al. observed that % LYM values decreased in rats exposed to sodium arsenite compared to the control group. According to our study, a significant decrease in the number of monocytes was observed in rats exposed to sodium arsenite. This decrease was valid for all groups except for the royal jelly group. These results are in line with the study conducted by him and his team. In addition, while the MID value in the Arc-R-100 group showed the lowest value in percentage terms, it was observed that these values gradually approached the control group with the increase in the amount of royal jelly in the Arc-R-200 group. This finding again refers to the potential of royal jelly to support the immune system.

Arsenic can negatively affect heme metabolism by binding to hemoglobin. This effect results in a decrease in hemoglobin levels inside red blood cells. Hemoglobin is a critical component that ensures the ability of red blood cells to transport oxygen. Due to its effect on hemoglobin, arsenic can impair the function of red blood cells and negatively affect oxygen transportation throughout the body. Therefore, it is important to monitor hematological parameters to understand and prevent arsenic-related health problems [30]. Arsenic exposure has been reported to contribute to anemia in humans and rodents by suppressing bone marrow function [31]. There appears to be a possible correlation between arsenic exposure and RBC and HGB levels. This relationship may be attributed to the reduced capacity of the bone marrow to produce red blood cells, leading to anemia in these groups [32]. Basher et al. [33] reported that HGB and RBC values decreased in rats exposed to sodium arsenite compared to the control group. In our study, HGB and RBC values decreased compared to the control group. There was a significant decrease in HGB level ($P<0.005$) between the R group and Arc-R-2 and between Arc-R-1 and Arc-R-2 groups. Although these decreases were not significant in the RBC parameter, they were parallel in terms of significance. Although the group given royal jelly alone (R) had the highest HGB and RBC counts, the combination of sodium arsenite and royal jelly and the increased dose of royal jelly caused a greater decrease in HGB and RBC counts. This may be because individuals administered sodium arsenic may have experienced anemia due to persistent high levels of this substance in their bloodstream, which may have triggered erythrocyte hemolysis and subsequent decrease in hemoglobin levels.

Hematocrit, also known as packed cell volume, is used to determine the percentage of red blood cells in whole blood. Automated methods calculate this value by multiplying the number of RBCs and the average cell volume, while manual methods involve measuring the hematocrit after centrifugation of a microcapillary tube filled with whole blood [7]. Hematocrit refers to the percentage of packed blood cells relative to the total blood volume. The formula HCT = (RBC x MCV)/10 is used to calculate hematocrit. Following research, it has been found that chronic exposure to low levels of arsenic can negatively affect the body's red blood cells, hematocrit, and hemoglobin levels [16]. There appears to be a discernible association between arsenic exposure and decreased RBC and HCT/PCV measurements [30]. Sharma and Rani observed that arsenic exposure significantly decreased HCT levels in albino rats. In this study, the lowest HCT level was observed only in the group given sodium arsenite. Recent research suggests that low HCT levels may indicate different health conditions. Low HCT values may be a result of conditions such as anemia, excessive fluid intake, renal failure, or chronic inflammatory diseases [34]. The highest HCT level was detected only in the royal jelly group, suggesting that royal jelly may increase hematocrit levels. In conclusion, in the study, hematocrit levels increased in royal jelly-treated groups but decreased in sodium arsenite-treated groups. This suggests that royal jelly may increase hematocrit levels and arsenic exposure may decrease hematocrit levels.

One of the important values in hematologic analysis is the Mean Platelet Volume and Platelet count. MPV shows the average size of platelets and often reflects platelet activity. These parameters are vital for understanding the functioning of the blood coagulation system and for diagnosing various hematological disorders [35]. Mean Platelet Volume determines the overall size of blood cells by measuring the size of platelets. These parameters are used in routine blood morphology tests and play an important role in assessing blood clotting ability [36]. In the study, it was found that the highest MPV rate was in the Arc group and the lowest MPV rate was in the Arc-R-200 group. Kalia et al. reported that arsenic exposure caused a decrease in platelet levels in their study [37]. The highest mean PLT value was observed in the Arc-R-100 and Arc-R-200 groups. The reason for this is that MPV is inversely proportional to platelet count under normal physiologic conditions. That is, the higher the platelet count, the lower the mean volume of platelets, and this is associated with maintaining blood clotting ability and maintaining a constant mass of platelets. [38]. This suggests a decrease in the average volume of platelets accompanying their increased production. In different pathological conditions, this normal physiological ratio can be disrupted. In particular, increased or abnormal platelet production, increased destruction of platelets, or the effect of activation factors on platelets can lead to changes in the ratios between MPV and PLT. These changes can be observed during hematologic analyses and can provide healthcare professionals with important information about the condition of blood cells.

5. CONCLUSION

Sodium arsenite exposure can cause hematologic changes in rats. These changes include an increase in white blood cells and decreased levels of red blood cells, hemoglobin, and hematocrit. An increase in granulocytes, a marked increase in lymphocytes, and a decrease in monocytes have also been observed. Arsenic exposure can also cause a decrease in hemoglobin and erythrocyte levels and a decrease in hematocrit levels. These findings emphasize that arsenic exposure can have serious effects on hematological parameters. It is suggested that royal jelly administration may influence these changes and in some cases may be protective against arsenic exposure. In particular, royal jelly may have the potential to protect the body against toxicity by increasing white blood cell count and lymphocyte count. Changes in mean platelet volume and platelet count were also observed and it is thought that royal jelly may affect these changes. Royal jelly may have protective effects against sodium arsenite toxicity and has been shown to have positive effects on the immune system. However, decreases in red blood cells, hemoglobin, and hematocrit levels have been noted and further research is needed. Further studies are also needed to identify the components of royal jelly that contribute to these positive effects.

The study did not show significant differences in hematologic parameters such as MID, RBC, MCHC, MCH, MCV, and PDW. However, decreases in %LYM, %MID, HCT, and PLT values and increases in WBC, GRA, and %GRA values were observed in all experimental groups. These results indicate that royal jelly may strengthen the immune system against the effects of harmful substances such as arsenic. The study also emphasizes the possible protective role of royal jelly against toxic effects. This suggests that royal jelly may provide positive effects on health and may be a valuable complementary food for those aiming to improve health. However, further research is needed to determine the appropriate royal jelly dosage and to understand the mechanisms in more depth. This information highlights the potential protective effects of royal jelly and its positive effects on the immune system.

REFERENCES

[1] Beltekin B, Demir N. International Journal of Food, Agriculture and Animal Sciences (IJFAA) Production and Some Bioactive Properties of Royal Jelly- A Review Arı Sütü Üretimi ve Bazı Biyoaktif Özellikleri - Derleme. 2022; 2(1): 38–46.

[2] Ramadan MF, Al-Ghamdi A. Bioactive Compounds and Health-Promoting Properties of Royal Jelly: A Review. J. Funct. Foods 2012; 4(1): 39–52.

[3] Fratini F, Cilia G, Mancini S, Felicioli A. Royal Jelly: An Ancient Remedy with Remarkable Antibacterial Properties. Microbiol. Res. 2016; 192: 130–141.

[4] Özbolat G, Tuli A. Ağır Metal Toksisitesinin İnsan Sağlığına Etkileri. Arşiv Kaynak Tarama Derg. 2016; 25(23783): 502–521.

[5] Seven T, Can B, Darende BN, Ocak S. Hava ve Toprakta Ağır Metal Kirliliği. Ulus. Çevre Bilim. Araştırma Derg. 2018; 1(2): 91–103.

[6] Başkan MB, Pala A. İçme Sularinda Arsenik Kirliliği : Ülkemiz Açisindan Bir Değerlendirme. Pamukkale Üniversitesi Mühendislik Bilim. Derg. 2009; 15(1): 69–79.

[7] Bolliger AP, Everds N. Haematology of the Mouse. Lab. Mouse 2012: 331–347.

[8] Kelada SNP, Aylor DL, Peck BCE, Ryan JF, Tavarez U, Buus RJ, Chesler DRM, Threadgill DW, Churchill GA, de Villena FPM, Collins FS. Genetic Analysis of Hematological Parameters in Incipient Lines of the Collaborative Cross. G3 Genes, Genomes, Genet. 2012; 2(2): 157–165.

[9] Lakna. DifferencACe Between Gram Positive and Gram Negative Bacteria Stunning Images of Cells Discover How Scientists Use Main Difference AC– Gram Positive vs Gram Negative Bacteria. Pediaa 2017; (April): 13.

[10] Kumar V. R. Robbins Temel Patoloji. İstanbul: Nobel 2003.

[11] Ola-Davies OE, Akinrinde AS. Acute Sodium Arsenite-Induced Hematological and Biochemical Changes in Wistar Rats: Protective Effects of Ethanol Extract of Ageratum Conyzoides. Pharmacognosy Res. 2016; 8(Suppl 1): S26.

[12] Mank V, Azhar W, Brown K. Leukocytosis. StatPearls 2023.

[13] Tigner A, Ibrahim SA, Murray I V. Histology, White Blood Cell. StatPearls 2022.

[14] Frandson RD, Wilke WL, Fails AD. Anatomy and Physiology of Farm Animals. 2009: 512.

[15] Linke B, Schreiber Y, Picard-Willems B, Slattery P, Nüsing RM, Harder S, Geisslinger G, Scholich K. Activated Platelets Induce an Anti-Inflammatory Response of Monocytes / Macrophages through Cross-Regulation of PGE 2 and Cytokines. 2017; 2017.

[16] Ciarrocca M, Tomei G, Palermo P, Caciari T, Cetica C, Fiaschetti M, Gioffrè PA, Tasciotti Z, Tomei F, Sancini A. Environmental and Biological Monitoring of Arsenic in Outdoor Workers Exposed to Urban Air Pollutants. Int. J. Hyg. Environ. Health 2012; 215(6): 555–561.

[17] Dacie J V, Lewis SM. Practical Haematology. 1995: 609–609.

[18] Kaya E, Şimşek H, Çakar F. Arı Sütünün Senesens ve Telomer Etkisi. In:. Sağlık Bilim. Alanında Uluslararası Araştırmalar IX. 2022; 83–95.

[19] Nikolić R, Krstić N, Jovanović J, Kocić G, Cvetković TP, Radosavljević-Stevanović N. Monitoring the Toxic Effects of Pb, Cd and Cu on Hematological Parameters of Wistar Rats and Potential Protective Role of Lipoic Acid and Glutathione. Toxicol. Ind. Health 2015; 31(3): 239–246.

[20] Ramadan MF, Al-Ghamdi A. Bioactive Compounds and Health-Promoting Properties of Royal Jelly: A Review. J. Funct. Foods 2012; 4(1): 39–52.

[21] Smedley PL, Kinniburgh DG. United Nations Synthesis Report on Arsenic in Drinking Water Developed on Behalf of the United Nations Administrative Committee on Cooperation Sub-Committee on Water Resources, with Active

Participation of UNICEF, UNIDO, IAEA, and the World Bank. Br. Geol. Surv. Wallingford 2001:1–390.
[22] Ohaeri CC, Eluwa MC. Abnormal Biochemical and Haematological Indices in Trypanosomiasis as a Threat to Herd Production. Vet. Parasitol. 2011; 177(3–4): 199–202.
[23] Kumari S, Rani S, Kumar A. Ameliorative Effect of Panax Quinquefolius on Sodium Arsenite Induced Toxicity in Charles Foster Rats. J. Appl. Nat. Sci. 2022; 14(4): 1532–1541.
[24] Sharma S, Rani S. Possible Protective Role of Aqueous Tomato Extract on Hemato-Biochemical Parameters against Sodium Arsenite Toxicity in Albino Rats. J. Pharm. Res. Int. 2022; 34(14B): 12–22.
[25] Lin A, Loré K. Granulocytes: New Members of the Antigen-Presenting Cell Family. Front. Immunol. 2017; 8(DEC): 1781.
[26] Blumenreich MS. The White Blood Cell and Differential Count. Clin. Methods Hist. Phys. Lab. Exam. 1990.
[27] Adebayo AH, Zeng GZ, Fan JT, Ji CJ, He WJ, Xu JJ, Zhang YM, Akindahunsi AA, Kela R, Tan NH. Biochemical, Haematological and Histopathological Studies of Extract of Ageratum Conyzoides L. in Sprague Dawley Rats. J. Med. Plants Res. 2010; 4(21): 2264–2272.
[28] Ewere EG, Okolie NP, Ndem JI, Oyebadejo SA, Ewere EG, Okolie NP, Ndem JI, Oyebadejo SA. Immunological and Hematological Effects of Irvingia Gabonensis Stem Bark in Sodium Arsenite-Exposed Rats. Https://Gsconlinepress.Com/Journals/Gscbps/Sites/Default/Files/GSCBPS-2021-0089.Pdf 2021; 15(1): 027–037.
[29] NHLBI, NIH. Lymphopenia - What Is Lymphopenia? Https//Www.Nhlbi.Nih.Gov/Health/Lymphopenia Date Accessed 2023-09-07 2022.
[30] Lu M, Wang H, Li XF, Lu X, Cullen WR, Arnold LL, Cohen SM, Le XC. Evidence of Hemoglobin Binding to Arsenic as a Basis for the Accumulation of Arsenic in Rat Blood. Chem. Res. Toxicol. 2004; 17(12): 1733–1742.
[31] Szymańska-Chabowska A, Antonowicz-Juchniewicz J, Andrzejak R. Some Aspects of Arsenic Toxicity and Carcinogenicity in Living Organism with Special Regard to Its Influence on Cardiovascular System, Blood and Bone Marrow. Int. J. Occup. Med. Environ. Health 2002; 15(2): 101–116.
[32] Ferland R, Gadahi JA, Saleha S, Ali Q. Histological and Haematological Disturbance Caused by Arsenic Toxicity in Mice Model. Pakistan J. Biol. Sci. 2008; 11(11): 1405–1413.
[33] Basher MK, Sarkar S, Mobarak MH, Islam MR. Inorganic Arsenic Exposure during Pregnancy Affects Post-Natal Growth, Blood Parameters, and Organ Development of Mice Offspring. J. Adv. Biotechnol. Exp. Ther. 2023; 6(2): 445–457.
[34] Kundrapu S, Noguez J. Laboratory Assessment of Anemia, vol. 83. Elsevier Inc. 2018.
[35] Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemona H, Dymicka-Piekarska V. Mean Platelet Volume (MPV): New Perspectives for an Old Marker in the Course and Prognosis of Inflammatory Conditions. Mediators Inflamm. 2019; 2019.
[36] Sikora J, Kostka B. Blood Platelets as Pharmacological Mode. Postępy Biol. Komórki 2005.
[37] Kalia K, Narula GD, Kannan GM, Flora SJS. Effects of Combined Administration of Captopril and DMSA on Arsenite Induced Oxidative Stress and Blood and Tissue Arsenic Concentration in Rats. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 2007; 144(4): 372–379.
[38] Thompson CB, Jakubowski JA. The Pathophysiology and Clinical Relevance of Platelet Heterogeneity. Blood 1988; 72(1): 1–8.